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Evaluation of High-Resolution Gas for the Determination of 2, 3, 7, 8-TCDD in Soil Sediment and Water (Journal Version)

Midwest Research Inst., Kansas City, MO

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16. ABSTRACT

An analytical protocol for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and total TCDDs in soil, sediment and aqueous samples using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) was evaluated through replicate analyses of fortified and unspiked soil and aqueous samples. The results of these analyses demonstrated that the method is useful for the determination of 2,3,7,8-TCDD and total TCDDs at concentrations ranging from 10 to 200 pg/g (ppt) in soil samples and from 100 to 2000 pg/l (ppq) in aqueous samples. Absolute recoveries of the method internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, ranged from 24 to 88%. Lower levels of detection can be achieved through a slight modification of the method with respect to initial sample size and/or final extract volume. Instrumental parameters that affect data quality, specifically HRGC column performance, mass resolution and mass measurement accuracy are presented.

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Evaluation of a High-resolution Gas Chromatography/High-resolution Mass Spectrometry Method for the Determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Soil and Water

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An analytical protocol for the determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and total TCDDs in soil, sediment, and aqueous samples using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) was evaluated through replicate analyses of fortified and unspiked soil and aqueous samples. The results of these analyses demonstrated that the method is useful for the determination of 2,3,7,8-TCDD and total TCDDs at concentrations ranging from 10 to 200 pg/g (ppt) in soil samples and from 100 to 2000 pg/l (ppq) in aqueous samples. Absolute recoveries of the method internal standard, ($^{13}C_{12}$-2,3,7,8-TCDD, ranged from 24 to 88%. Lower levels of detection can be achieved through a slight modification of the method with respect to initial sample size and/or final extract volume. Instrumental parameters that affect data quality, specifically HRGC column performance, mass resolution and mass measurement accuracy are presented.

INTRODUCTION

Methods for the determination of low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and total TCDDs in soil/sediment and aqueous samples are required to address the impact of the release of these compounds to the environment. Mass spectrometry with various sample introduction techniques has been used as the cornerstone to achieve the necessary specificity and sensitivity for TCDD analyses. High-resolution mass spectrometry (HRMS) can provide the specificity necessary to confirm the presence of TCDDs in environmental and biological matrices by eliminating much of the chemical noise resulting from isobaric interferences.

Early analytical methods incorporating HRMS for determining TCDDs have included high-resolution dual-ion monitoring with direct probe introduction or packed-column gas chromatography (PGC).[1–11] These methods were sensitive for the entire group of TCDDs, but fairly non-specific for 2,3,7,8-TCDD owing to a lack of efficient sample preparation and clean-up procedures and because of the low separating power of the direct-inlet and PGC sample introduction techniques. These early methods for TCDD analysis relied heavily on HRMS to differentiate chromatographic responses arising from potential interferences such as PCBs, DDE, etc., from those due to TCDDs.

The specificity and sensitivity of the HRMS analytical methods were advanced by state-of-the-art developments in sample preparation/clean-up procedures and the introduction of high-resolution gas chromatographic (HRGC) columns.[12–15]

The emphasis for the application of HRMS for final analysis for 2,3,7,8-TCDD and total TCDDs declined as a result of further development of sample preparation/clean-up procedures. Specifically, the introduction of high-performance liquid chromatography (HPLC) separation schemes provided the capability to determine specific TCDD isomers using PGC combined with low-resolution mass spectrometry (LRMS).[16–20] Also, the introduction of carbon-based clean-up procedures resulted in more specificity in determining 2,3,7,8-TCDD by LRMS as the result of the more efficient removal of the potential interferences, such as PCBs and other polychlorinated aromatics.

Analytical methods based on HRGC/LRMS were then developed in support of Superfund programs for identifying 'hot spots' and supporting clean-up activities of Superfund sites (such as Times Beach, Missouri). These methods, through the use of improved sample preparation/clean-up procedures, have the potential for providing low parts-per-trillion (ppt) to low parts-per-

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quadrillion (ppq) detection limits for TCDDs in relatively clean soil and water samples, respectively. However, because these methods are based on LRMS, they are not applicable for determination of TCDDs in samples having a significant level of interferences in the matrix.

Because of the complexity of the issues raised by the presence of 2,3,7,8-TCDD and the possible legal implications and impending regulatory actions, analytical methods that provide more specificity are necessary. In response to these needs, an analytical method[21] was developed which incorporates what are considered to be the best features of several analytical procedures.[22–29] This method, combined with the capability of HRMS, provides a means for achieving the necessary specificity and sensitivity for the confirmation of 2,3,7,8-TCDD at low concentration levels.

The primary objective of this study, therefore, was to evaluate the protocol[21] that has been designed for use as a routine method in support of regional and national needs of the United States Environmental Protection Agency (EPA). The experimental approach and results of the analyses completed for the evaluation of the protocol and a discussion of critical method parameters are presented.

EXPERIMENTAL

Sample sources

Five water matrices were obtained or generated to demonstrate the analytical method performance. These aqueous samples consisted of distilled water, the influent and effluent of a sewage treatment facility, an industrial waste effluent, and an aqueous leachate from a soil sample highly contaminated with 2,3,7,8-TCDD.

The industrial wastewater was very acidic (pH < 1) and was contaminated with high levels of PCB and other chlorinated aromatic compounds. The aqueous leachate was prepared by mixing 10 g of the highly contaminated soil sample with approximately 4 l of distilled water for at least 24 h prior to subsampling for analysis.

Four soil samples were provided by the Environmental Monitoring Support Laboratory, Las Vegas, Nevada (EMSL-LV). The soil samples were originally collected from sites contaminated with 2,3,7,8-TCDD. The indigenous levels of 2,3,7,8-TCDD in these soil samples were estimated from previous analyses to range from 50 to 1700 pg/g.

Analytical standards and reagents

The 2,3,7,8-TCDD was obtained from the EPA Quality Assurance Materials Bank (Research Triangle Park, North Carolina) (7.87 ± 0.78 μg/ml in isooctane). The 1,2,3,4-; 1,3,6,8-/1,3,7,9-; 1,2,3,7-/1,2,3,8-; and 1,2,7,8-, 1,4,7,8-TCDD isomers were obtained as the neat compounds (approximately 1 mg quantities) from Cambridge Isotope Laboratories (Woburn, Massachusetts). The recovery standard ($^{13}C_{12}$-1,2,3,4-TCDD, 50 ± 5 μg/ml in *n*-nonane) and the quantification standard ($^{13}C_{12}$-2,3,7,8-TCDD, 50 ± 5 μg/ml in *n* nonane) were obtained from Cambridge Isotope Laboratories.

Calibration standards were prepared with the two ^{13}C-labeled TCDD standards and the unlabeled 2,3,7,8-TCDD in tridecane. Standards were prepared at 2.5, 5.0, 10.0, 20.0 and 40.0 pg/μl of 2,3,7,8-TCDD and the recovery standard, $^{13}C_{12}$-1,2,3,4-TCDD. The internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, was added at 10.0 pg/μl to each of the calibration standards. In addition, an HRGC column performance mixture containing the 1,3,6,8-, 1,3,7,9-, 1,4,7,8-, 2,3,7,8-, 1,2,3,7-, 1,2,3,4-, and 1,2,8,9-TCDD and both the recovery and internal standards was prepared.

All solvents for extraction and clean-up were obtained from Burdick & Jackson as 'distilled-in-glass' quality. Tridecane (99%) was obtained from Aldrich. The preparation of the acidic alumina (Bio-Rad, AG-4, 100–200 mesh), the silica gel (Kieselgel 60, 70-230 mesh), the sulfuric-acid-modified silica gel, sodium sulfate, potassium carbonate, Celite 545, silanized glass wool, and Carbopak C (Supelco, 80-100 mesh) are described in the detailed analytical protocol.[21]

Sample preparation

The general sample extraction and cleanup procedures are presented in Fig. 1.

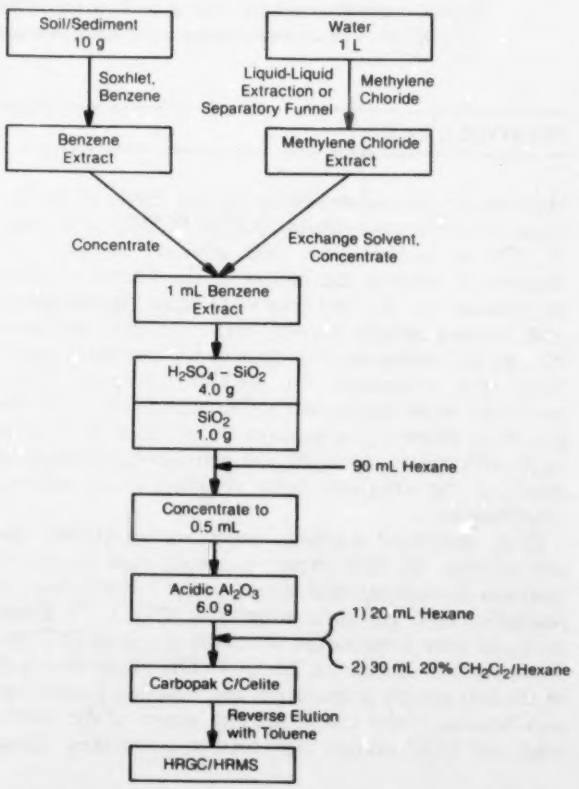

Figure 1. Extraction, clean-up, and analysis steps in the refined protocol.

Extraction. Soil samples were weighed (1–10 g portions) as received and were spiked with 1.5 ml acetone containing 500 pg of the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, and were mixed with approximately 10 g anhydrous sodium sulfate. The mixture was placed in a Soxhlet apparatus on a bed of glass wool and was extracted continuously with 200-250 ml benzene for 24 h. The extract was poured through a filter funnel containing anhydrous sodium sulfate and was concentrated in a Kuderna-Danish (K-D) apparatus to approximately 5 ml. The extract was further concentrated to approximately 1 ml with a gentle flow of purified nitrogen. For those samples for which the 2,3,7,8-TCDD concentration was estimated to be greater than 200 pg/g from previous analyses, approximately 1 g portions were analyzed following the same extraction procedure. A single aliquot of each of the soil matrices was fortified with additional TCDD isomers (1,3,6,8-, 1,3,7,9-, 1,2,3,7-, 1,2,3,8-, 1,2,3,4-, 1,2,7,8-, and 1,2,8,9-TCDD) at levels ranging from 53 to 2500 pg/g.

All aqueous samples with the exception of the soil leachate and duplicate aliquot of the industrial wastewater were fortified with 2,3,7,8-TCDD at concentrations ranging from 250 to 1000 pg/l. One aliquot of each of the distilled water, influent and effluent wastewaters, and the industrial wastewater samples was fortified with the additional TCDD isomers at levels ranging from approximately 500 pg/l to over 3000 pg/l. The aqueous samples (1 l) were spiked with 1.5 ml acetone containing 500 pg $^{13}C_{12}$-2,3,7,8-TCDD and were extracted sequentially with three 60 ml portions of methylene chloride. The organic extracts were combined and passed through anhydrous sodium sulfate. The extracts were subsequently concentrated in a K-D apparatus. Benzene (50 ml) was added to each extract before concentrating to a final volume of 1 ml with purified nitrogen.

Extract clean-up. The concentrated benzene extract was added to the top of a chromatographic column (1 cm × 10 cm) packed (from bottom to top) with a glass-wool plug, a 1 cm layer of anhydrous sodium sulfate and potassium carbonate (1:1), 1.0 g silica gel, and 4.0 g 40% (w/w) sulfuric-acid-impregnated silica gel. The sulfuric-acid-impregnated silica gel was prepared by adding two parts (by weight) of concentrated sulfuric acid to three parts of extracted and activated silica gel. The extract was eluted from the silica gel column with 90 ml hexane, and the eluate was concentrated to 0.5 ml by using K-D apparatus and nitrogen blowdown.

The extract was transferred onto a chromatographic column (1 cm × 30 cm) packed from bottom to top with a glass-wool plug, 6.0 g acidic alumina (Bio-Rad, AG-4) Soxhlet extracted with methylene chloride for 6 h and activated overnight at 190 °C, and a 1 cm layer of anhydrous sodium sulfate. The column was then washed with 20 ml hexane, which was discarded, and eluted with 30 ml 20% (v/v) methylene chloride in hexane.

The TCDD isomers were isolated from the eluate on a carbon-based chromatographic column consisting of a mixture (approximately 340 mg) of 18% Carbopak C (Supelco)/Celite 545 (Fischer Scientific) packed as a 2.0–2.5 cm bed in the center of a disposable 5 ml pipette. This carbon column was precleaned with a series of solvents which included 2 ml toluene, 1 ml methylene chloride/methanol/benzene (75:20:5), 1 ml cyclohexane/methylene chloride (1:1) and 2 ml hexane. The 30 ml of eluate from the acidic alumina column was passed through the Carbopak C/Celite 545 column at approximately 0.5 ml/min. The column was then eluted sequentially with two 1 ml portions of hexane, 1 ml cyclohexane in methylene chloride (1:1), and 1 ml methylene chloride/methanol/benzene (75:20:5). The column was then inverted, and the TCDD fraction was eluted with 6 ml toluene which was collected in a concentrator tube.

The toluene fraction was warmed to 60 °C in a constant temperature water bath, and was concentrated to approximately 1 ml by using a gentle stream of purified nitrogen. The extract was transferred to a 1 ml micro vial, and the volume was further reduced with a stream of nitrogen to approximately 100 µl. The concentrator tube was washed with three 200 µl portions of 1% toluene in methylene chloride which were added to the micro vial. Tridecane (50 µl) was added to the micro vial, and the extracts were refrigerated until just prior to analysis.

HRGC/HRMS analysis

The refrigerated extracts were allowed to reach room temperature, and 5 µl of the recovery standard solution (100 pg/µl of $^{13}C_{12}$-1,2,3,4-TCDD in isooctane) was added. The sample extracts were concentrated at room temperature, using a gentle stream of nitrogen, to the final volume of 50 µl. The sample extracts and concentration calibration standards were analyzed with a Carlo Erba Mega Series gas chromatograph that was coupled to a Kratos MS50TC double-focusing mass spectrometer operating in the selected ion monitoring (SIM) mode. The gas chromatograph/mass spectrometer interface was a direct connection of the HRGC column to the ion source via a heated (240 °C) interface oven. A Finnigan 2300 Incos data system was used for data acquisition and processing. The HRMS operating conditions were: accelerating voltage, 8000 V; electron energy, 70 eV; source temperature, 280 °C; and mass resolution, 10 000 (10% valley definition). GC was performed using a CP-Sil 88 (50 m × 0.2 mm, 0.2 µm film thickness, Chrompack) column with helium (linear velocity 25 cm/s) as the carrier gas. Samples were injected (2 µl) through a split/splitless injector held at 270 °C with splitless time of 45 s, a split flow of 30 ml/min, and a septum purge of 6 ml/min. Separation was achieved using a temperature program starting at 200 °C isothermal for 1 min followed by programming to 240 °C at 4 °C/min.

The mass spectrometer was tuned daily using perfluorokerosene (PFK) to yield a resolution of at least 10 000 (10% valley definition) and optimal response at *m/z* 254.986. This step was followed by mass calibration of a linear accelerating voltage scan beginning at the magnet set position of *m/z* 254. Other voltage scans from the same data file were then used to establish and document both the mass resolution at *m/z* 316.983 and

the mass measurement accuracy at *m/z* 330.979. No concentration calibration solutions or sample extracts were analyzed until it was demonstrated that the mass measurement accuracy at *m/z* 330.979 was better than 5 ppm and the resolution at *m/z* 316.983 was better than 10000.

Following calibration, the experiment descriptor was updated to reflect the new calibration. Five analytical masses, 258.930, 319.897, 321.894, 331.937 and 333.934, were monitored by scanning ~m/10000 amu or approximately 30 mmu over each mass. These masses correspond to (M + 2) − COCl, M and M + 2 for TCDD and M and (M + 2) for $^{13}C_{12}$-TCDD, respectively. The *m/z* 280.983 ion from PFK was included as a magnet lock mass because it is the most abundant PFK ion within the range of *m/z* 255 to 334; it therefore permits the use of low partial pressures of PFK, minimizing PFK interferences at the analytical masses. The total cycle time was kept to 1 s.

Prior to initiating the analysis of the sample extracts, a full calibration curve was established by performing triplicate analyses of the concentration calibration standards HRCC1 through HRCC5. Criteria for successful calibration required that the average response factor for the 2,3,7,8-TCDD relative to the $^{13}C_{12}$-2,3,7,8-TCDD (RRF(I)) and the $^{13}C_{12}$-2,3,7,8-TCDD relative to the $^{13}C_{12}$-1,2,3,4-TCDD (RRF(II)) had relative standard deviations (RSD) of ⩽20% at each concentration level. Furthermore, the grand mean response factor encompassing the entire concentration range was also required to have an overall RSD of ⩽20%.

The HRGC column performance was demonstrated daily through the analysis of a mixture of TCDD isomers. This mixture included the 1,3,6,8-TCDD and 1,2,8,9-TCDD, which are the first and last eluting TCDD isomers, as well as the 2,3,7,8-TCDD and the potentially coeluting isomers 1,2,3,4-TCDD, 1,4,7,8-TCDD, 1,2,3,7-TCDD and 1,2,3,8-TCDD. The mixture also contained $^{13}C_{12}$-2,3,7,8-TCDD, which was used to verify the retention time of the unlabeled 2,3,7,8-TCDD. Figure 2 presents an example of the HRGC/HRMS data from the analysis of this column performance mixture. Column performance was monitored by determining the height of the highest valley between 2,3,7,8-TCDD and the closest eluting TCDD isomers and dividing that by the height of the 2,3,7,8-TCDD response. Chromatographic performance yielding separation with a valley of 25% or less was required in order to consider the analysis an isomer-specific determination of 2,3,7,8-TCDD.

Before proceeding with the routine analysis of sample extracts, the analyst was required on a daily basis to (a) verify that the HRGC column provides the resolution required for isomer-specific determination for 2,3,7,8-TCDD, (b) document that the mass resolution and mass measurement accuracy are ⩾10000 and ⩽5 ppm, respectively, and (c) demonstrate, by analysis of the calibration standard containing 5.0 pg/μl of 2,3,7,8-TCDD, that the relative response factors were within ±20% of the grand means established initially from the replicate analyses of the concentration calibration standards.

The analysis of the sample extracts was initiated after successfully completing these steps. The analyst was also required to document the mass resolution at the end of each analysis day. Failure to meet the mass resolution criteria at the end of the day invalidated any results obtained on that day.

Quantification

Quantification was achieved by application of isotope-dilution methodology based upon the $^{13}C_{12}$-2,3,7,8-TCDD that was added to the original sample. Calculation of percentage recovery of that isotope diluent is achieved by addition of the second isotopically labeled

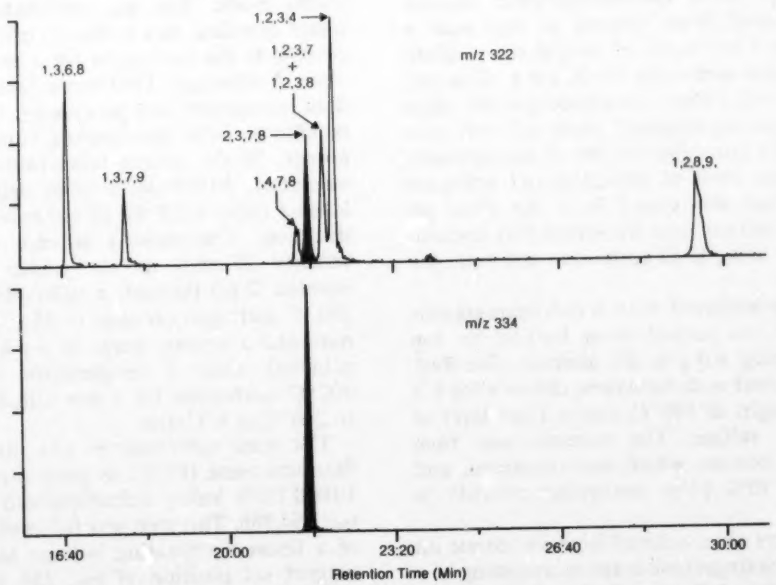

Figure 2. Example mass chromatograms resulting from the analysis of the HRGC column performance mixture showing the isomer-specific chromatographic performance and the elution window for TCDDs.

TCDD ($^{13}C_{12}$-1,2,3,4-TCDD) to the final extract as a 'recovery standard'. Before quantifying a response as 2,3,7,8-TCDD or other TCDD isomer, the following qualitative criteria[21] were applied. (i) Retention time for unlabeled 2,3,7,8-TCDD must be within −1 to +3 s of that for the isotopically labeled internal standard. Retention times of other TCDDs must fall within the RT window established by the GC column performance check mixture. (ii) Ion current responses for *m/z* 258.930, 319.897 and 321.894 of the unlabeled TCDDs must maximize simultaneously (±1 scan), and all ion current intensities must be at least 2.5 times the noise level. (iii) The response ratios for *m/z* 319.897/321.894 and for *m/z* 331.937/333.934 must be between 0.67 and 0.90 (0.77 theoretical). (iv) Ion current responses for *m/z* 331.937 and 333.934 of ^{13}C-labeled TCDDs must maximize simultaneously (±1 s). (v) Recovery of the internal standard ($^{13}C_{12}$-2,3,7,8-TCDD) must be between 40 and 120%.

Interlaboratory analysis. Selected (soil and aqueous) samples were analyzed at both the Midwest Research Institute (MRI) and the Quality Assurance Laboratory at the University of Nevada–Las Vegas (QASL/UNLV). Concentration calibration standards and spiking solutions of the quantification and recovery standards and the unlabeled TCDD isomers were split between the two laboratories.

Fortified field blanks. Before preparing any field samples, the laboratories had to demonstrate that the method yielded recoveries within the bounds of 40–120%. This was accomplished by analyzing duplicate blank 10 g solid and 1 l aqueous samples. Pre-extracted sand and distilled water were selected as the matrices for use as field blanks. One sample of each matrix was fortified with 1 ng 2,3,7,8-TCDD and was taken through the entire extraction and clean-up procedure. The final extract was fortified with $^{13}C_{12}$-2,3,7,8-TCDD that was used to calculate the absolute method recovery for 2,3,7,8-TCDD. The second field blank for each matrix was spiked with 1,2,3,4-TCDD and was carried through all analytical procedures to document background levels of TCDDs that were introduced as artifacts due to sample preparation procedures or analytical reagents. The final extracts of these samples were fortified with $^{13}C_{12}$-1,2,3,4-TCDD.

RESULTS

Initial instrument calibration

The results from the triplicate analysis of each of the calibration standards are shown in Table 1. The consistency of the relative response factors (RRF) determined for unlabeled 2,3,7,8-TCDD versus the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, and the RRF of the internal standard versus the recovery standard, $^{13}C_{12}$-1,2,3,4-TCDD, over the calibration range demonstrated that the HRGC/HRMS system maintains a linear response for 2,3,7,8-TCDD and $^{13}C_{12}$- 1,2,3,4-TCDD from 2.5 to 40 pg/μl.

Table 1. Summary of initial instrument calibration[a]

2,3,7,8-TCDD Calibration	RRF (I)[b]		RRF (II)[c]	
Standard (pg/μl)	Mean	RSD (%)	Mean	RSD (%)
2.5	0.776	2.9	2.24	2.4
5.0	0.848	2.0	1.99	13.0
10.0	0.970	0.5	1.56	1.9
20.0	0.949	1.7	1.50	1.1
40.0	0.987	2.2	1.46	3.9
Overall mean	0.906	9.4	1.75	19.3

[a] Data summarized from triplicate analysis of each calibration standard.
[b] RRF (I) = relative response factor for 2,3,7,8-TCDD relative to the $^{13}C_{12}$-2,3,7,8-TCDD.
[c] RRF (II) = relative response factor for $^{13}C_{12}$-2,3,7,8-TCDD relative to the $^{13}C_{12}$-1,2,3,4-TCDD.

The precision (RSD) of RRF(I) for the unlabeled 2,3,7,8-TCDD congener was less than 3.0% for each of the five individual concentration calibration solutions and was less than 10% over the entire calibration range. Triplicate analysis of an additional standard prepared at 1.0 pg/μl of 2,3,7,8-TCDD demonstrated RRF(I) values within the established curve. However, the responses at the 1.0 pg/μl level did not routinely meet the qualitative criteria requiring that the *m/z* 320/322 ion ratio be between 0.67 and 0.90. The precision measurements for RRF(II), although within calibration criteria (≤20% RSD), were more variable than for RRF(I). This larger variability of RRF(II) is attributed to the higher level of chemical noise in the *m/z* 332 and 334 channels, which complicates integration of the chromatographic peaks.

Analysis of aqueous samples

The results from the replicate analyses of the aqueous samples are presented in Table 2. Fortification levels of 2,3,7,8-TCDD ranged from 250 pg/l for distilled water up to 1000 pg/l in the effluent wastewater matrix. The average accuracy for 2,3,7,8-TCDD, defined as the ratio of the amount of 2,3,7,8-TCDD found to the amount spiked, ranged from 99 to 104% for the replicate analyses of the distilled water and of the influent and effluent matrices from the sewage treatment facility. The results from the analysis of the fortified industrial wastewater indicate an average concentration of 1410 ppq at the 500 ppq spike level. Duplicate analyses of the unfortified industrial wastewater indicated that the 2,3,7,8-TCDD background concentration was 620 ± 24 ppq.

The soil leachate was not fortified since this sample was generated from a matrix that was highly contaminated with 2,3,7,8-TCDD. Initial analyses of this matrix were unsuccessful because of a high level of suspended solids and responses at *m/z* 332 that interfered with the internal and recovery standards. Successful analysis of the soil leachate was achieved after the sample was centrifuged for several minutes to remove the suspended solids. The duplicate analysis of the clarified sample yielded an average concentration of 27,600 ± 990 ppq (see Table 2).

Table 2. Accuracy of the HRGC/HRMS method for the determination of TCDD isomers in aqueous matrices

TCDD analyte	Distilled Water			Influent Wastewater			Effluent Wastewater			Industrial Wastewater		
	Spike (pg)	Measured (pg)	Accuracy (%)	Spike (pg)	Measured (pg)	Accuracy (%)	Spike (pg)	Measured (pg)	Accuracy (%)	Spike (pg)	Measured (pg)	Accuracy (%)
2,3,7,8[a]	250	248	99	500	524	104	1,000	1,040	104	0	616[c]	—
$^{13}C_{12}$-2,3,7,8[b]	500	410	82	500	355	71	500	455	91	500	290	58
1,3,6,8	460	512	111	920	582	63	1,840	502	27	920	ND[d]	0
1,3,7,9	210	395	190	420	690	164	840	766	91	420	ND	0
1,2,3,7/1,2,3,8	420	403	96	840	940	112	1,680	1,860	111	840	ND	0
1,2,3,4	610	616	101	1,220	1,280	105	2,440	1,840	75	1,220	ND	0
1,2,7,8	770	840	109	1,540	1,790	116	3,080	3,430	111	1,540	586	38
1,2,8,9	300	328	109	600	690	115	1,200	1,330	111	600	ND	0

[a] The values for 2,3,7,8-TCDD are the averages of triplicate analysis of each sample matrix. The precision of the measurements for 2,3,7,8-TCDD ranged from 2.5 to 8.2%.
[b] The values for $^{13}C_{12}$-2,3,7,8-TCDD are the averages for triplicate analysis of each sample matrix. The precision of the absolute recovery of the $^{13}C_{12}$-2,3,7,8-TCDD ranged from 4.1 to 40%.
[c] Measured value corrected for indigenous 2,3,7,8-TCDD content (averaged 616 ± 24 pg/l).
[d] Not detected.

The precision of the analytical results for all aqueous samples ranged from 3.6% for the soil leachate to 16% for the fortified industrial wastewater. The absolute recovery of the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, ranged from an average of 24% for the fortified industrial wastewater to 87% for the soil leachate. (With the exception of the analysis of the industrial wastewater, the absolute recovery of the internal standard did not appreciably affect the accuracy of the measurement for 2,3,7,8-TCDD.)

It is interesting to note the differences in the absolute recoveries of the internal standard for the fortified and unfortified industrial wastewater samples. The fortified wastewater was analyzed as duplicate 1 l samples and yielded absolute recoveries of the internal standard ranging from 20 to 29%. Considerably better recoveries (average of 58%) were noted for the unfortified matrix which was analyzed as duplicate 500 ml samples. These results imply that the relative amounts of coextractants from the matrix have a substantial effect on the method performance.

One portion of each of the aqueous samples, with the exception of the soil leachate, was spiked with additional TCDD isomers. Table 2 also summarizes the spike levels and percentage recoveries of these isomers from the different matrices. Data for the 1,3,6,8- and 1,3,7,9-TCDD isomers when compared with the other isomers demonstrate that the early eluting isomers are most affected by the sample matrix. Recoveries of the other isomers spiked into the distilled water and into the influent and effluent wastewaters were quite good. The other individual isomers were measured at 75–116% of the original spike values. The absolute recovery of the internal standard varied from 71 to 91% in these three matrices.

The additional TCDD isomers were not recovered efficiently from the industrial wastewater matrix. As noted in Table 2, the absolute recovery of the internal standard was only 20%. The only other isomer besides the 2,3,7,8-TCDD detected and quantified in this matrix was the 1,2,7,8-TCDD (accuracy = 38%).

Analysis of soil samples

The results of the replicate analyses of the four soil samples are summarized in Table 3. Since these samples were collected from areas known to be contaminated

Table 3. Accuracy of the HRGC/HRMS method for the determination of TCDD isomers spiked into soil matrices

TCDD analyte	Soil no. 1			Soil no. 2			Soil no. 3			Soil no. 4		
	Spike (pg)	Measured (pg)	Accuracy (%)	Spike (pg)	Measured (pg)	Accuracy (%)	Spike (pg)	Measured (pg)	Accuracy (%)	Spike (pg)	Measured (pg)	Accuracy (%)
2,3,7,8[a]	—	15.4	—	—	33.4	—	—	1,000	—	—	2030	—
$^{13}C_{12}$-2,3,7,8[b]	500	345	69	500	290	58	500	415	83	500	440	88
1,3,6,8	92	ND (9.2)[c]	0	130	29.0	22	660	333	50	1560	383	25
1,3,7,9	42	15.2	36	60	51.1	85	300	635	212	710	367	52
1,2,3,7/1,2,3,8	84	61.8	74	120	125	104	600	518	86	1430	825	58
1,2,3,4	120	54.1	45	170	118	69	880	695	79	2070	855	41
1,2,7,8	150	147	98	220	252	115	1110	1170	105	2620	2330	89
1,2,8,9	60	63.9	107	84	100	119	430	463	108	1020	952	93

[a] The values for 2,3,7,8-TCDD are the averages of the triplicate analysis of each sample matrix. The precision of the triplicate analysis of each of these matrices ranged from 11 to 25%.
[b] The values for $^{13}C_{12}$-2,3,7,8-TCDD are the averages of the triplicate analysis of each sample matrix. These values are reported as absolute recovery (%) rather than method accuracy (%). The precision of the absolute recoveries of the $^{13}C_{12}$-2,3,7,8-TCDD for each of these matrices ranged from 12 to 27%.
[c] ND, not detected. The value in parentheses reflects the estimated detection limit.

with 2,3,7,8-TCDD, the subsamples were not fortified with additional 2,3,7,8-TCDD. The data for the replicate analyses of the four soil samples document that the method provides quantitative data in the low ppt concentration range. The soil samples numbered 3 and 4 were actually analyzed as approximately 1.0 g portions. This demonstrates the applicability of this analytical method for extending the performance of the analytical method from concentrations as low as 10 pg/g (10 ppt) to as high as 2000 pg/g (2 ppb).

The precision of the replicate analyses of the soil samples varies somewhat more than was found for the aqueous samples. This may be attributed to differences in the matrices which are due to homogeneity, soil characteristics, etc. Homogeneity of the soil matrix is a significant factor, expecially when the requirement for analyzing 1.0 g samples is considered.

The absolute recovery of the internal standard ranged from an average of 58% for soil 2 up to 88% for soil 4. A comparison of the precision (RSD) range of the internal standard recoveries and the precision of the measured 2,3,7,8-TCDD concentrations for each of the soil matrices demonstrates the utility of the isotope dilution principle.

A portion of each of the four soil matrices was spiked with additional TCDD isomers. The results from these analyses are given in Table 3. As noted with the analyses of the fortified water samples, the recoveries for the 1,3,6,8-TCDD and the 1,3,7,9-TCDD are quite variable. The accuracy of the measurements for the remaining isomers ranges from an average of 58% for the 1,2,3,4-TCDD up to 107% for the 1,2,8,9-TCDD.

Interlaboratory comparison

Two of the soil samples and the industrial wastewater were subsampled and supplied to a second laboratory for the determination of 2,3,7,8-TCDD. The same sample preparation and HRGC/HRMS protocol were followed by both laboratories. All concentration calibration standards and spiking solutions were prepared from a common source to ensure that the interlaboratory study focused on the performance of the analytical protocol. Each laboratory was required to meet the specific calibration, column resolution, mass resolution and mass accuracy criteria identified in the experimental section.

The results of the replicate analyses completed by each laboratory are presented in Table 4. The results for the analysis of the aqueous sample are most consistent between the laboratories. The average aqueous concentration determined by Laboratory 1 was 616 pg/l (3.9% RPD) as compared to 622 pg/l (18.4% RPD) determined by Laboratory 2. Laboratory 1 reported average concentrations of 15.4 pg/g for soil 1 and of 1000 pg/g for soil 3 as compared to average concentrations of 10.3 pg/g and 899 pg/g, respectively, reported by Laboratory 2. The differences in the average values for the soil samples may in part be attributed to inhomogeneity of the original sample. The homogeneity of the matrix is especially important in considering the results from soil 3 since only a 1.0 g portion of this sample was analyzed. Also it should be noted that the comparison of the results for soil 1 may be indicative of the method performance limit as the 2,3,7,8-TCDD concentration approaches the demonstrated lower level of quantification.

Table 4. Comparison of analytical data from an interlaboratory study

Matrix	Laboratory 1[a]	Laboratory 2[b]
Industrial Wastewater	604, 628 (pg/l)	565, 680 (pg/l)
Soil no. 1	18.2, 15.1, 12.9 (pg/g)	10.9, 8.8, 11.7, 9.9 (pg/g)
Soil no. 3	937, 785, 1,280 (pg/g)	870, 928 (pg/g)

[a] Analyses completed with a Kratos MS-50TC using a 50 m CP-Sil 88.
[b] Analyses completed with a VG-ZAB using a 60 m SP-2330.

Fortified method blanks

As part of the overall quality assurance/quality control (QA/QC) program identified in the HRGC/HRMS protocol, fortified method blanks were analyzed to demonstrate (a) that the extraction and clean-up procedures provide recoveries of the 2,3,7,8-TCDD within the criteria of greater than 40% specified in the protocol, and (b) that the reagents are free from contamination with TCDD isomers.

The analyses of the fortified method blanks at the outset of the study demonstrated that the recoveries of 2,3,7,8-TCDD and 1,2,3,4-TCDD ranged from 65% to 79%. No detectable levels of other TCDD isomers were found in this preliminary study. The fortified method blanks analyzed with the actual samples resulted in recoveries of 29% and 83%. However, these analyses demonstrated some interferences arising from 1,3,6,8- and 1,3,7,9-TCDD at levels ranging from less than 10 to approximately 50 pg/g. Previous studies involving evaluation of the cleanup procedure indicated that these isomers are associated with the acidic alumina cleanup.[21] Additional experience with this method has demonstrated that the background contributions of 1,3,6,8- and 1,3,7,9-TCDD can be minimized by preelution of the acidic alumina prior to the application of the sample extract.[31]

The results of the analyses of the fortified method blanks and of the samples not spiked with the 1,3,6,8- and 1,3,7,9-TCDD isomers were plotted versus time elapsed since preparation of the acidic alumina. The first set of fortified method blanks was taken through the acidic alumina column 7 days after preparation of the alumina. Although responses were observed at m/z 320 and 322 at the retention time for these isomers, the ion ratios did not indicate the presence of the compounds. Since the detectable levels were well below 10 pg/g of alumina, the analyses of the real samples were initiated. The data for the fortified blanks and field samples taken through the acidic alumina column from 14 to 30 days after the alumina activation indicated that the contamination by the 1,3,6,8- and 1,3,7,9-TCDD isomers apparently occurs over time in one particular oven which was used to activate the alumina. Background contamination by 1,3,6,8- and 1,3,7,9-TCDD isomers has also been recognized in other labor-

atories.[29] In that study, background TCDD levels were attributed to the use of phenolic-based floor cleaners in the laboratory.

DISCUSSION

Several factors were identified that are critical to the successful application of the analytical protocol for determining total TCDDs and specifically of 2,3,7,8-TCDD. These factors include both sample preparation steps (specifically clean-up) and selection of the appropriate instrumental parameters (HRGC column selection, mass calibration, mass measurement accuracy and mass resolution), and are discussed below.

Sample preparation

One of the primary considerations during the initial development of the analytical method was a streamlined approach for sample preparation. The sample clean-up procedures were originally designed to avoid excessive handling of the sample extract. Sample extracts of approximately 1 ml volume in benzene were put directly through the sequence of clean-up columns. Concentration of the eluate was not necessary until the appropriate fraction (toluene) was collected from the Carbopak C/Celite column.

An initial evaluation[21] of the method resulted in very low recoveries (typically less than 50%) of the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, and the accuracy and precision of duplicate sample analyses were poor (typically less than 50% recovery). After reviewing the data, it was apparent that the problems resulted from poor chromatographic separation in the clean-up columns. Further evaluation of the clean-up procedure demonstrated that the presence of as little as 1% benzene in the extract which was directed into the acidic alumina column resulted in significant losses (greater than 50%) of TCDDs, especially of the early eluting isomers 1,3,6,8- and 1,3,7,9-TCDD.

In order to avoid these losses, the analyst must ensure that benzene has been effectively removed from the extract by repeated solvent exchange with hexane and by concentration to approximately 0.5 ml volume before fractionation on the acidic alumina. Alternatively, this solvent exchange step might be included prior to chromatography on the acid-modified silica gel column, although the analyst must be aware of potential problems of solubility that might be associated with the total organic extractable content of the original sample. An example of the effect of coextractants from the sample matrix on analyte recovery is the analysis of the industrial wastewater (Table 2). The absolute recovery of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD was markedly improved (greater than 80%) for spiked reagent blanks after the procedures for ensuring complete solvent exchange had been implemented. Although the required concentration step increases the time required for the total sample preparation effort, the accuracy of the method is greatly improved.[21]

Concentration of sample extracts to the final volume of 50 µl must be accomplished at ambient temperature, as discussed in the method protocol. Loss of as much as 50% of the TCDD has occurred when the concentration to the final volume is completed at elevated temperatures.[31]

Selection of the HRGC column

Three different HRGC columns were evaluated in the course of this project: SP-2330 (60 m × 0.24 mm); DB-5 (60 m × 0.22 mm); and CP-Sil 88 (50 m × 0.22 mm). By evaluating the mass spectra of the bleed from each column at 240–250 °C, it became apparent that the condition of the column and the associated column background may be the limiting factor in achieving the desired detection limit for this method. The DB-5 column provided the least amount of background at 250 °C. The CP-Sil 88 column appeared to bleed less than the SP-2330 column, and it permitted lower levels of quantification to be achieved because of reduced background contribution.

The chromatographic performance afforded by these columns is a further issue since the column best suited for low detection limits, DB-5, cannot always meet the 25% valley chromatographic performance criteria. Both the SP-2330 and CP-Sil 88 columns can readily resolve the 2,3,7,8-TCDD from the other TCDD isomers. However, with reference to the bleed considerations discussed above, the 50 m CP-Sil 88 column was selected for the analyses in our laboratory.

The HRGC column performance can degrade very quickly if proper injection techniques are not used. Specifically, the SP-2330 and CP-Sil 88 phases are polar and very sensitive to O_2 and will decompose rapidly at 200 °C if any trace of O_2 is present. Therefore, the common practice of using 1 µl of air to flush the syringe and to effect reproducible injections must be avoided, since even that small amount of air per injection can cause the column performance to degrade in less than one week of continued column use (40–50 injections).

Mass measurement accuracy

Mass measurement accuracy was measured relative to PFK *m/z* 254.986 by applying the mass correction value, Δm, to the entire spectrum which yields an error of 0 ppm at *m/z* 254.986. In this way, it was possible to meet routinely the 5 ppm mass measurement accuracy criterion at *m/z* 330.979. Because the lock mass is used to maintain magnet alignment and will ultimately control the accuracy of the SIM mass selection, it may be preferable to perform mass measurements relative to the SIM lock mass (*m/z* 280.983) rather than *m/z* 254.986. However, simultaneous monitoring of mass measurement accuracy at *m/z* 330.979 relative to *m/z* 254.986 and *m/z* 280.983 (lock mass) revealed no significant differences in mass measurement accuracy.

Mass resolution

Mass resolution checks were carried out for *m/z* 316.983 by plotting the mass peak profile with the Incos PROF

program. However, it was found that the computer-generated value for resolution was frequently higher than the value measured manually from the same profile and the value determined by the use of the peak matching unit. Thus, the manually determined resolution, which was computed as $m/\Delta m$ (at 5% peak height) was reported in this study. Closer inspection of the PROF source code revealed that resolution is computed via a statistical method, not as the traditional $m/\Delta m$ at 5% height. Incos users should therefore be aware of this discrepancy, because the computer-generated value for mass resolution can be as much as 20% higher than the actual value.

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Notice

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